Site Directed Mutagenesis

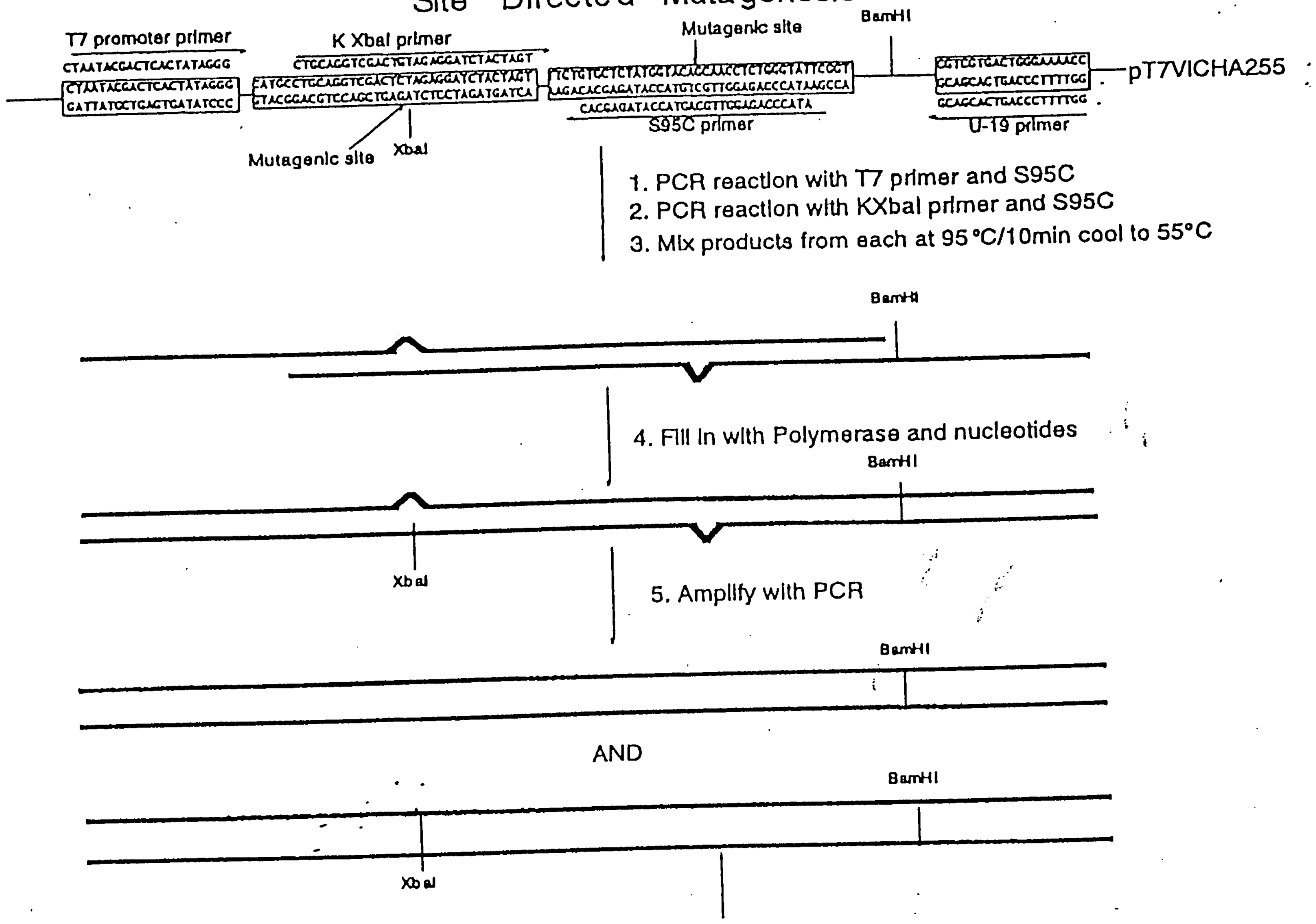
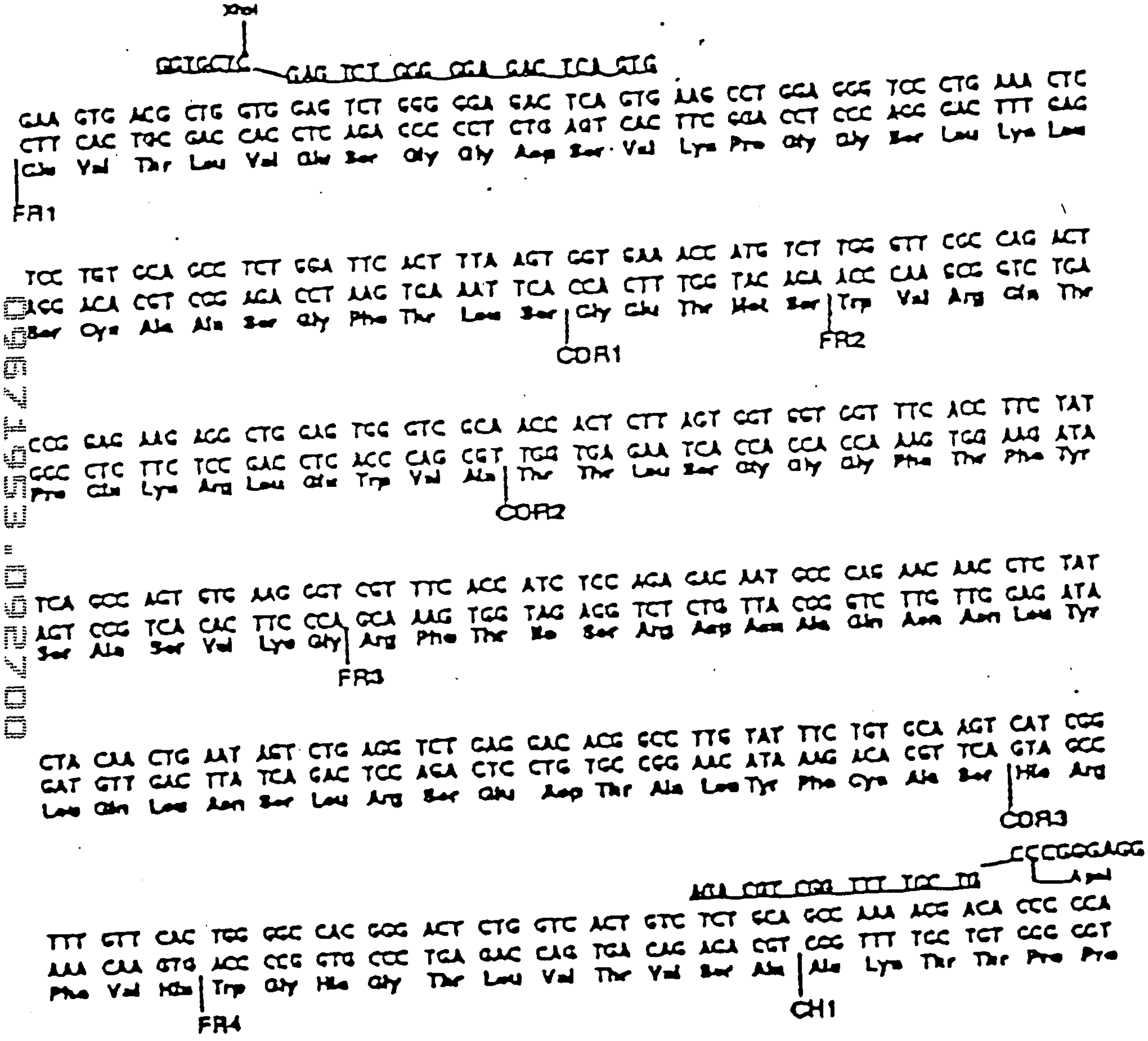
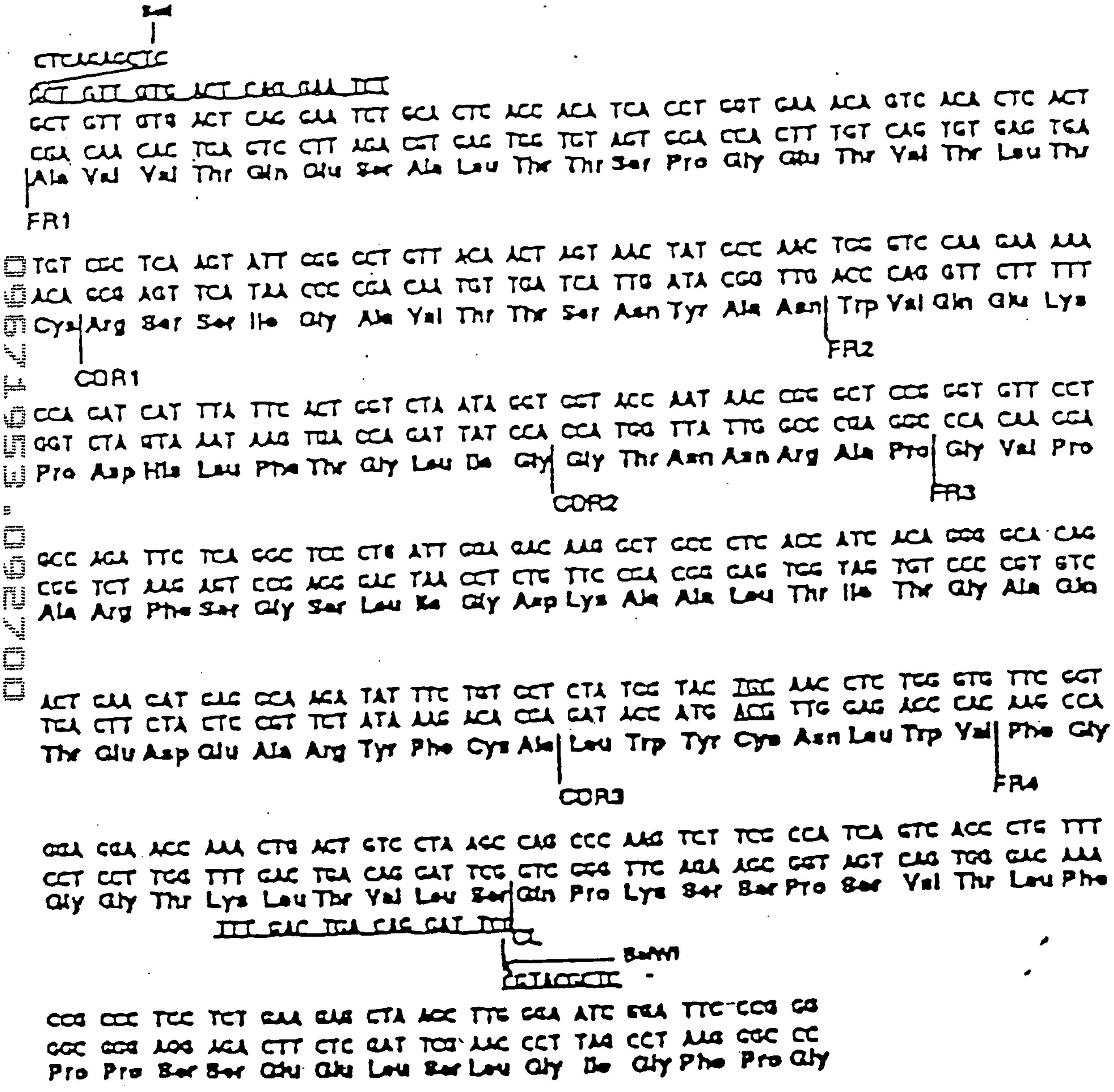


Fig. 1



XhoI
GCTGCTC GAG TCT GGG GGA GAC TCA GTG
GAA GTG ACG CTG GTG GAG TCT GGG GGA GAC TCA GTG AAG CCT GGA GGG TCC CTG AAA CTC
CTT CAC TGC GAC CAC CTC AGA CCC CCT CTG AGT CAC TTC GGA CCT CCC AGG GAC TTT GAG
Glu Val Thr Leu Val Glu Ser Gly Gly Asp Ser Val Lys Pro Gly Gly Ser Leu Lys Leu
FR1
TCC TGT GCA GCC TCT GGA TTC ACT TTA AGT GGT GAA ACC ATG TCT TGG GTT CGC CAG ACT
AGG ACA CGT CGG AGA CCT AAG TGA AAT TCA CCA CTT TGG TAC AGA ACC CAA GCG GTC TGA
Ser Cys Ala Ala Ser Gly Phe Thr Leu Ser Gly Glu Thr Met Ser Trp Val Arg Gln Thr
CDR1
FR2
CCG GAG AAG AGG CTG GAG TGG GTC GCA ACC ACT CTT AGT GGT GGT GGT TTC ACC TTC TAT
GGC CTC TTC TCC GAC CTC ACC CAG CGT TGG TGA GAA TCA CCA CCA CCA AAG TGG AAG ATA
Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Thr Leu Ser Gly Gly Gly Phe Thr Phe Tyr
CDR2
TCA GCC AGT GTG AAG GGT CGT TTC ACC ATC TCC AGA GAC AAT GCC CAG AAC AAC CTC TAT
AGT CGG TCA CAC TTC CCA GCA AAG TGG TAG AGG TCT CTG TTA CGG GTC TTG TTG GAG ATA
Ser Ala Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn Asn Leu Tyr
FR3
CTA CAA CTG AAT AGT CTG AGG TCT GAG GAC ACG GCC TTG TAT TTC TGT GCA AGT CAT CGG
GAT GTT GAC TTA TCA GAC TCC AGA CTC CTG TGC CGG AAC ATA AAG ACA CGT TCA GTA GCC
Leu Gln Leu Asn Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr Phe Cys Ala Ser His Arg
CDR3
CCCGGGAGG
ApaI
AGA CGT CGG TTT TGC TT
TTT GTT CAC TGG GGC CAC GGG ACT CTG GTC ACT GTC TCT GCA GCC AAA ACG ACA CCC CCA
AAA CAA GTG ACC CCG GTG CCC TGA GAC CAG TGA CAG AGA CGT CGG TTT TGC TGT GGG GGT
Phe Val His Trp Gly His Gly Thr Leu Val Thr Val Ser Ala Ala Lys Thr Thr Pro Pro
FR4
CH1

Fig. 2



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GCT GTT GTG ACT CAG GAA TCT GCA CTC ACC ACA TCA CCT GGT GAA ACA GTC ACA CTC ACT
CGA CAA CAC TGA GTC CTT AGA CGT GAG TGG TGT AGT GGA CCA CTT TGT CAG TGT GAG TGA
Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu Thr

TGT CGC TCA ACT ATT CGG GCT GTT ACA ACT AGT AAC TAT GCC AAC TGG GTC CAA GAA AAA
ACA GCG AGT TCA TAA CCC CGA CAA TGT TGA TCA TTG ATA CGG TTG ACC CAG GTT CTT TTT
Cys Arg Ser Ser Ile Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys

CCA GAT CAT TTA TTC ACT GGT CTA ATA GGT GGT ACC AAT AAC CGG GCT CCC GGT GTT CCT
GGT CTA GTA AAT AAG TGA CCA GAT TAT CCA CCA TGG TTA TTG GCC CGA GGG CCA CAA GGA
Pro Asp His Leu Phe Thr Gly Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro

GCC AGA TTC TCA GGC TCC CTG ATT GGA GAC AAG GCT GCC CTC ACC ATC ACA GGG GCA CAG
CGG TCT AAG AGT CCG AGG GAC TAA CCT CTG TTC CGA CGG GAG TGG TAG TGT CCC CGT GTC
Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln

ACT GAA GAT GAG GCA AGA TAT TTC TGT GCT CTA TGG TAC TGC AAC CTC TGG GTG TTC GGT
TGA CTT CTA CTC CGT TCT ATA AAG ACA CGA GAT ACC ATG ACG TTG GAG ACC CAC AAG CCA
Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Cys Asn Leu Trp Val Phe Gly

GGA GGA ACC AAA CTG ACT GTC CTA AGC CAG CCC AAG TCT TCG CCA TCA GTC ACC CTG TTT
CCT CCT TGG TTT GAC TGA CAG GAT TCG GTC GGG TTC AGA AGC GGT AGT CAG TGG GAC AAA
Gly Gly Thr Lys Leu Thr Val Leu Ser Gln Pro Lys Ser Ser Pro Ser Val Thr Leu Phe
            TTT GAC TCA CAG GAT TCG CA
                                    CGTACGCTC

CCG CCC TCC TCT GAA GAG CTA AGC TTG GGA ATC GGA TTC CCG GG
GGC GGG AGG AGA CTT CTC GAT TCG AAC CCT TAG CCT AAG GGC CC
Pro Pro Ser Ser Glu Glu Leu Ser Leu Gly Ile Gly Phe Pro Gly
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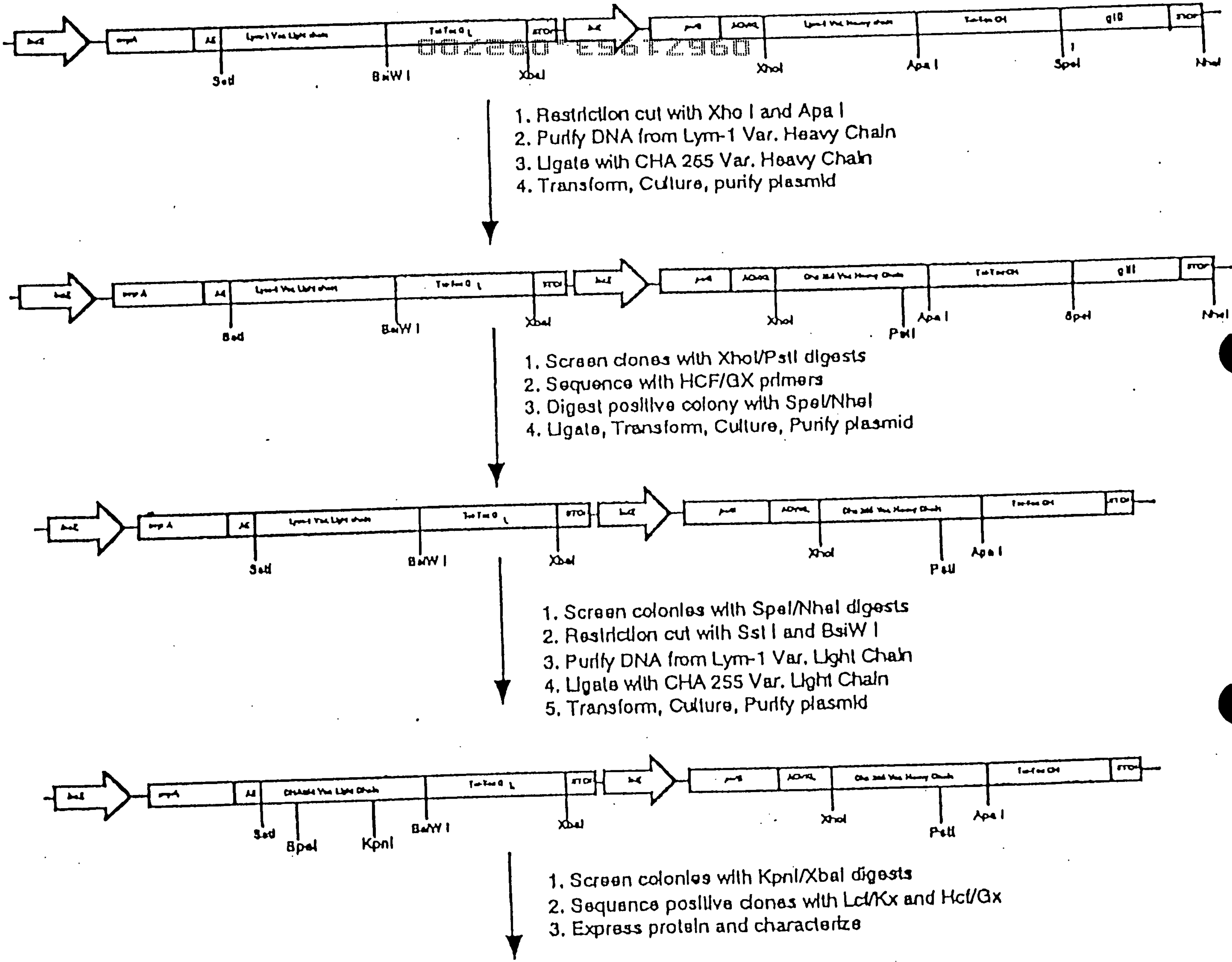
Fig. 3



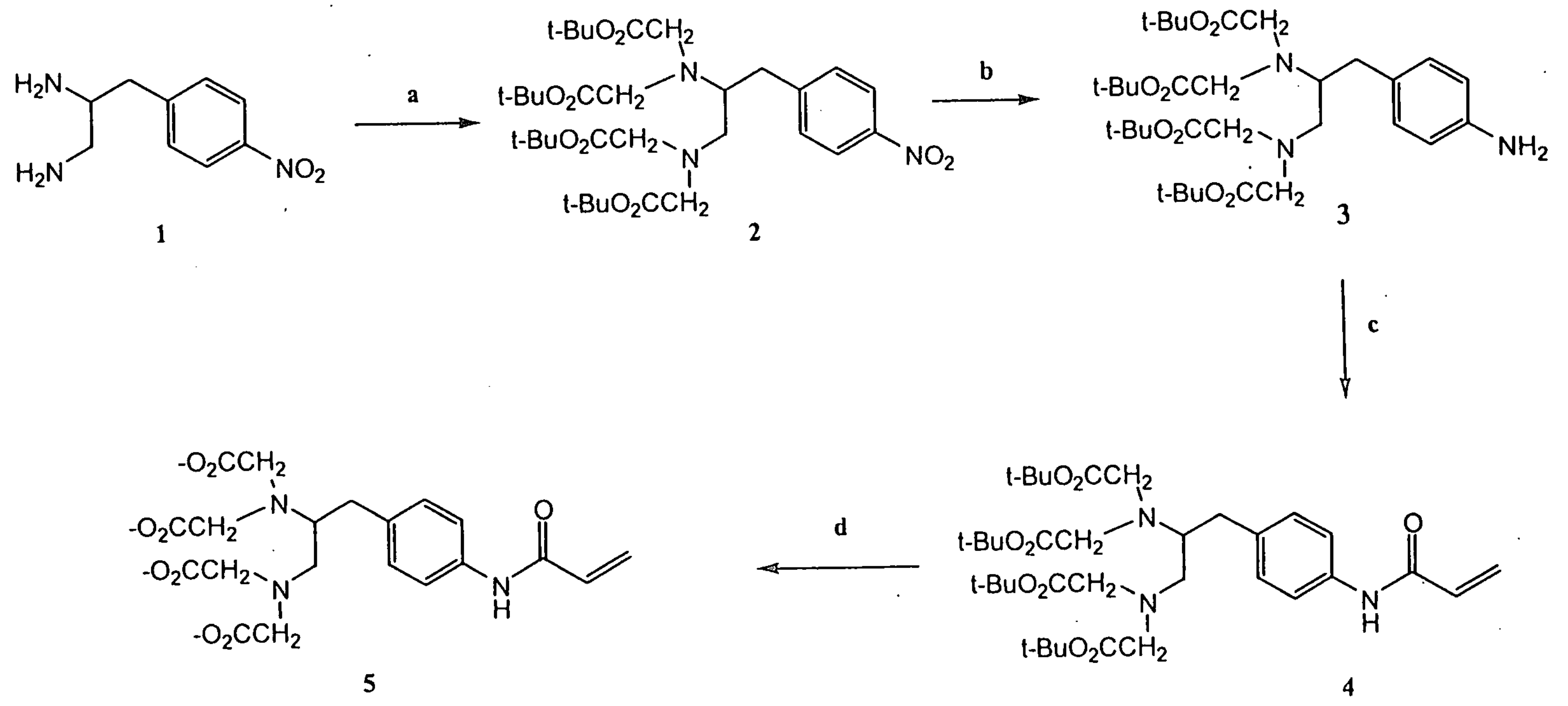
1. Restriction cut with Xho I and Apa I
2. Purify DNA from Lym-1 Var. Heavy Chain
3. Ligate with CHA 255 Var. Heavy Chain
4. Transform, Culture, purify plasmid

1. Screen clones with XhoI/PstI digests
2. Sequence with HCF/GX primers
3. Digest positive colony with SpeI/NheI
4. Ligate, Transform, Culture, Purify plasmid

1. Screen colonies with SpeI/NheI digests
2. Restriction cut with SstI and BsiW I
3. Purify DNA from Lym-1 Var. Light Chain
4. Ligate with CHA 255 Var. Light Chain
5. Transform, Culture, Purify plasmid

1. Screen colonies with KpnI/XbaI digests
2. Sequence positive clones with Lcf/Kx and Hcf/Gx
3. Express protein and characterize

Fig. 4



a. $BrCH_2CO_2t\text{-}Bu$, DIPEA, KI, DMF ; **b.** H_2, Pd/C, MeOH ; **c.** acryloyl chloride, DIPEA, CH_2Cl_2 ; **d.** TFA.

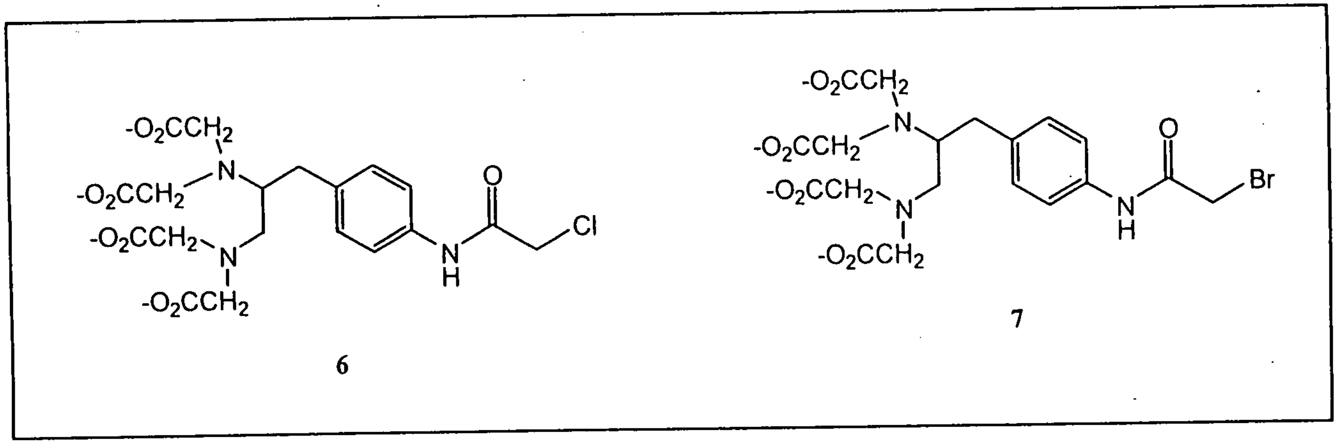


Fig. 5

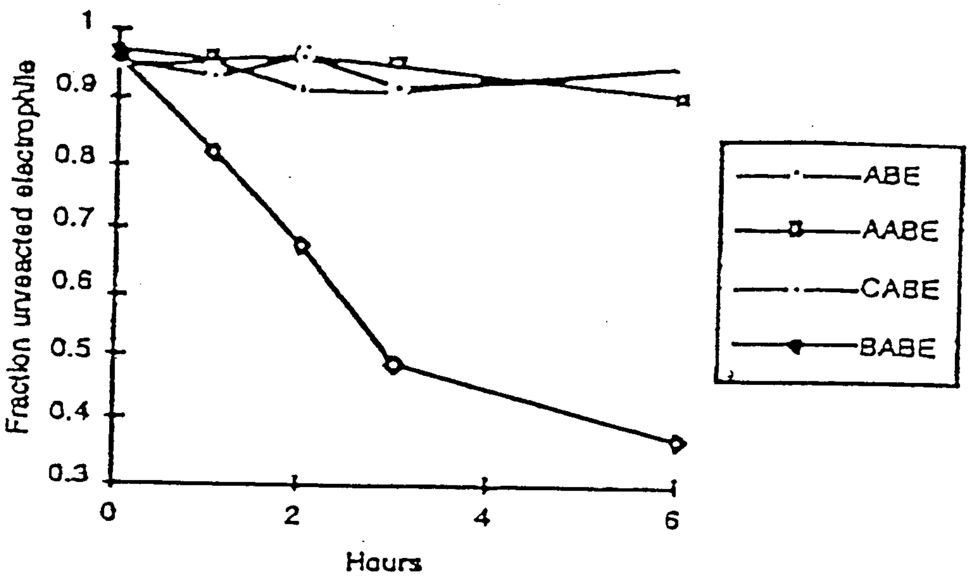

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Fraction unreacted electrophile
0
2
4
6
Hours
ABE
AABE
CABE
BABE

Fig. 6

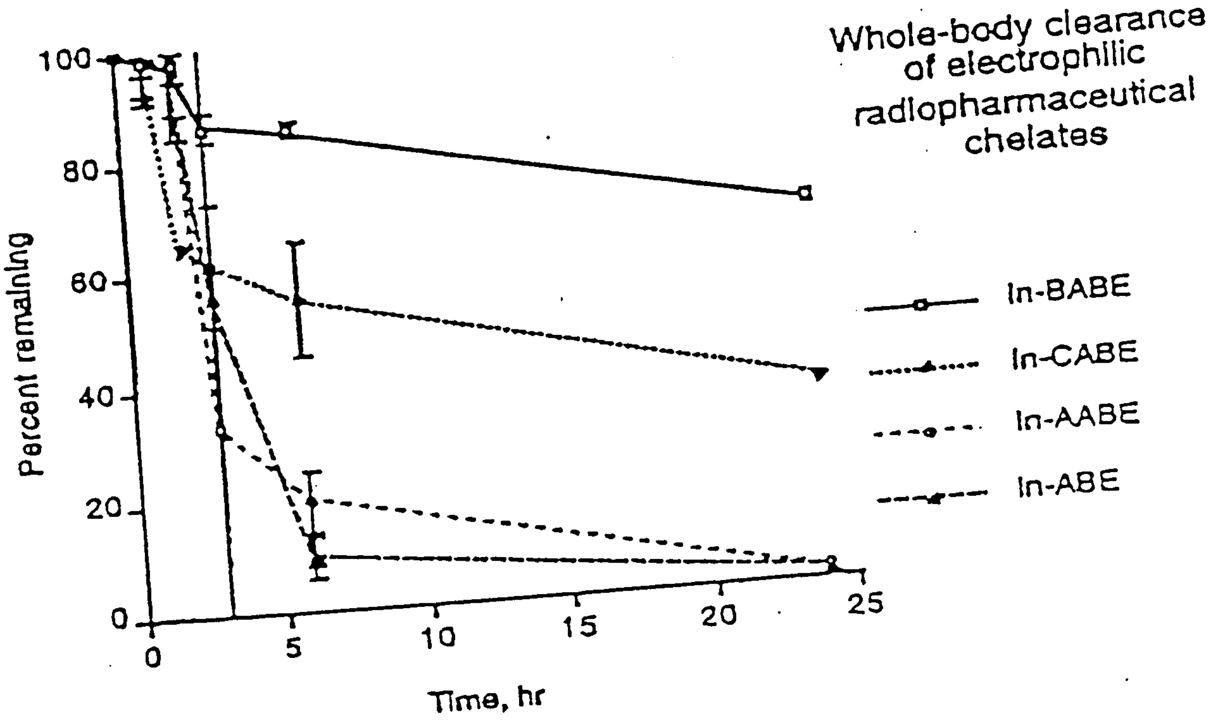

Whole-body clearance of electrophilic radiopharmaceutical chelates
100
80
60
40
20
0
Percent remaining
0
5
10
15
20
25
Time, hr
In-BABE
In-CABE
In-AABE
In-ABE

Fig. 7

AGATCTGAAGTGACGCTGGTGGAGTCTAGGGGAGACTCAGTGAAGCCTGGAGGGTT
CCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTAAGTGGTGAAACCATGTCTTG
GGTTCGCCAGACTCCGGAGAAGAGGCTGGAGTGGGTCACAACCACTCTTAGTGGTG
GTGGTTTCACCTTCTATTCAGCCAGTGTGAAGGGTCGTTTCACCATCTCCAGAGACA
ATGCCCAGAACAACCTCTATCTACAACTGAATAGTCTGAGGTCTGAGGACACGGCCT
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GGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGG
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CACCATCACCATTGA

Fig. 8

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GCTCCGGGTGTTCCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTC
ACCATCACAGGGGCACAGACTGAAGATGAGGCAAGATATTTCTGTGCTCTATGGTA
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GGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC
TGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG
GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGG
ACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGAC
TACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGYTYGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

Fig. 9

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>ACCATCACAGGGGCACAGACTGAAGATGAGGCAAGATATTTCTGTGCTCTATGGTA
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CTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT
GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAG
GACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
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CCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

Fig. 10

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CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGYTYGC
CCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

Fig. 11

RSAVVTQESALTTSPGETVTLTCRSSIGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPG
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FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSXPVTKSFNRGEC

Fig. 12

RSAVVTQESALTTSPGETVTLTCRSSIGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPG
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FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSXPVTKSFNRGEC

Fig. 13

RSAVVTQESALTTSPGETVTLTCRSSIGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPG
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FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSXPVTKSFNRGEC

Fig. 14

RSEVTLVESRGDSVKPGGFLKLSCAASGFTLSGETMSWVRQTPEKRLEWVTTTLSGGGF
TFYSASVKGRFTISRDN
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GGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSRLYFLSSVVTVPFNSLGTQTYICNVNHKPSNTKVD
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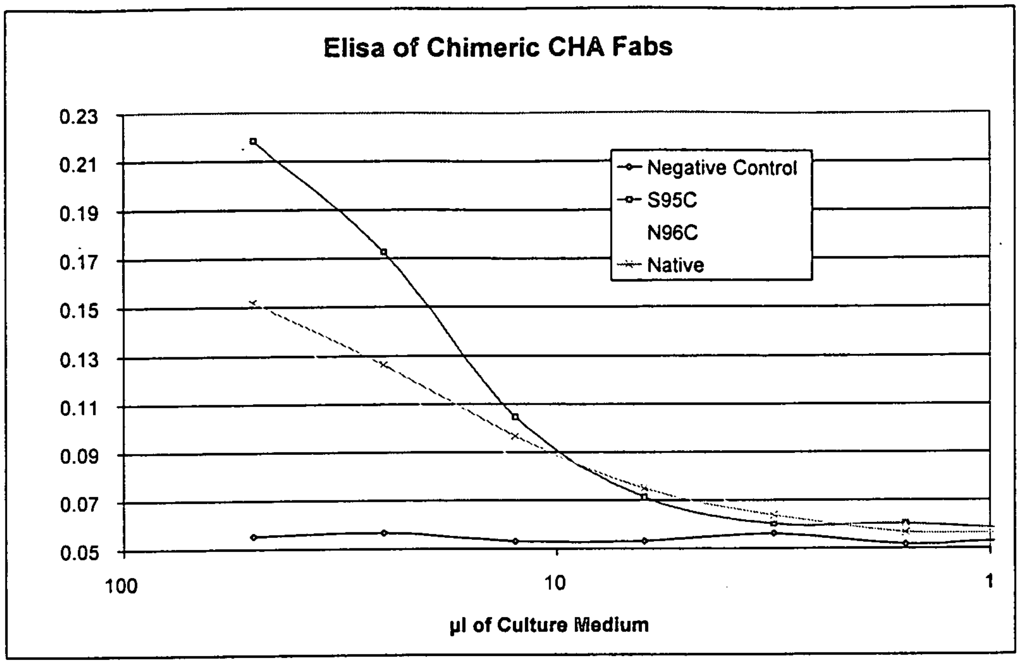
Fig. 15


Elisa of Chimeric CHA Fabs
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0.21
0.19
0.17
0.15
0.13
0.11
0.09
0.07
0.05
100
10
1
µl of Culture Medium
Negative Control
S95C
N96C
Native

Fig. 16

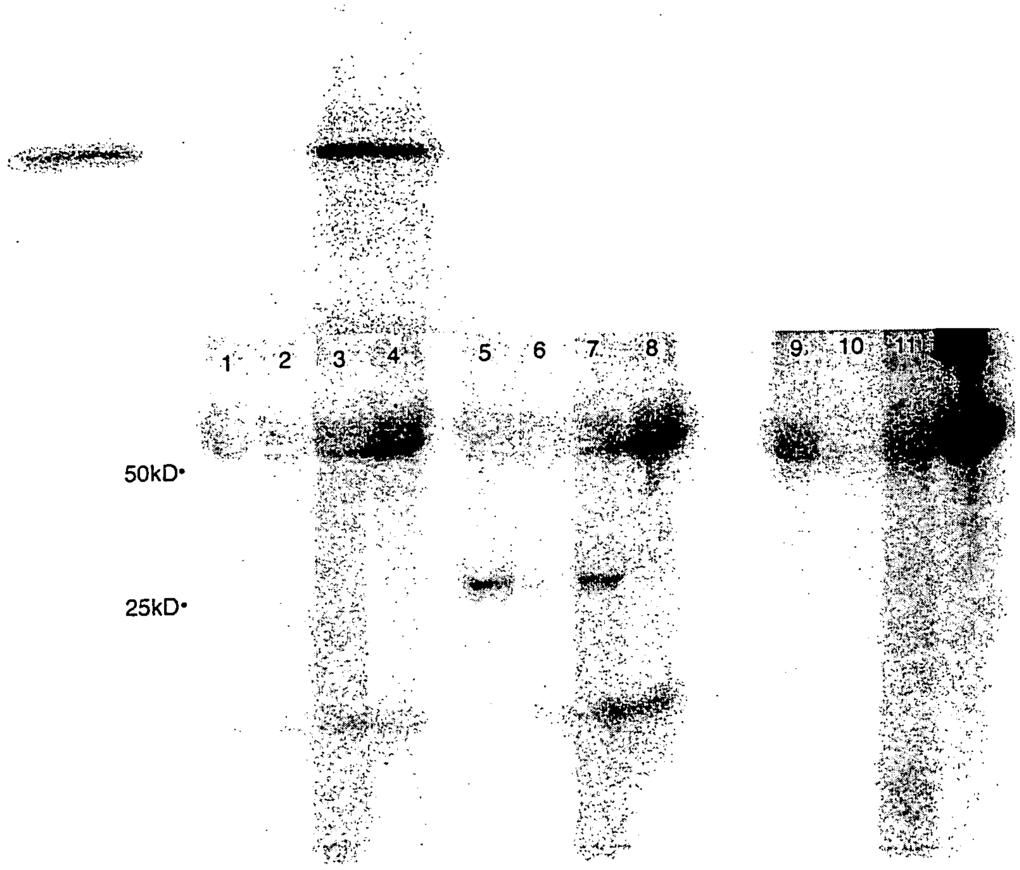

Fig. 17

Fig. 18